ENVIRONMENTAL EPIDEMIOLOGY

Effect of Environmental Chemicals on Human Health

By

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HS-1710 (Revised November 16, 1994)

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ADVANCES IN CHEMISTRY SERIES 241

Environmental Epidemiology

Effects of Environmental Chemicals on Human Health

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California Department of Health Services

Developed from a symposium sponsored
by the Division of Environmental Chemistry, Inc.
at the 203rd National Meeting
of the American Chemical Society,
San Francisco, California,
April 5–10, 1992

American Chemical Society, Washington, DC 1994

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Estimating Malathion Doses in California's Medfly Eradication Campaign Using a Physiologically Based Pharmacokinetic Model

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Physiologically based pharmacokinetic (PB-PK) models are designed to simulate the body as a series of tissue compartments, across which a chemical is absorbed, distributed, metabolized, and excreted in accord with pharmacokinetic rate laws. Well-constructed PB-PK models can be powerful tools for interpretation of biomarker data. This chapter demonstrates such use in estimating the absorbed malathion doses in subjects potentially exposed during an urban pesticide application. Single urine samples were collected from subjects within 48 h of a potential exposure for determination of malathion dicarboxylic acid, one of the pesticide's major metabolites. Subjects also responded to a questionnaire that provided a brief description of the circumstances and timing of their exposure. This PB-PK simulation suggests that for the adults (70 kg) and the children (14–34 kg), the highest absorbed doses were 1.3 and 0.4 mg, respectively.

CHARACTERIZING CHEMICAL EXPOSURES IN A STUDY POPULATION is one of the most difficult challenges in environmental epidemiology, a fact that has been emphasized in a number of chapters in this volume. The utility of biomarkers as probes of chemical exposure has been widely appreciated and,

0065-2393/94/0241-0189$08.00/0

as a result, the technology for measuring markers in biological samples has advanced significantly. In particular, analytical methods of determining xenobiotic metabolites in biological fluids (i.e., biomarkers of internal dose) are increasingly sensitive and reliable. Successful application of the new assays in epidemiological studies is not straightforward, however, because exact knowledge of the concentration of a biomarker in a sample does not translate to a dose (the parameter of greatest interest to the epidemiologist). Physiologically based pharmacokinetic (PB-PK) models are designed to treat the body as a series of tissue compartments, across which a chemical is absorbed, distributed, metabolized, and excreted in accord with pharmacokinetic rate laws. Well-constructed PB-PK models can be powerful tools for interpretation of biomarker data, as demonstrated here in estimating absorbed malathion doses in subjects allegedly exposed to aerial sprays during an urban pesticide application.

Malathion (*S*-1,2-bis[ethoxycarbonyl]ethyl *O,O*-dimethyl phosphorodithioate) is an insecticide that has been used widely in the United States to control mosquitoes, flies, and household insects since the early 1960s. This insecticide is a member of the organophosphorous group and, as such, can cause acute cholinergic reactions in humans at high dosages. Malathion is readily metabolized by microsomal enzymes through oxidation of the P=S bond to P=O, followed by hydrolysis of the phosphate ester. However, the predominant metabolic pathway in humans is deesterification of the ethyl succinate esters; two of its major urinary metabolites in humans are the monocarboxylic acid (MCA) and dicarboxylic acid (DCA). Gallo and Lawryk (*1*) recently provided a thorough review of the toxicology and metabolism of this pesticide in mammals.

As part of the recent Mediterranean fruit fly (Medfly) eradication campaign in California, aerial sprays of malathion mixed with a corn-syrup protein bait were applied repeatedly (up to a dozen times) over portions of southern California during the fall of 1989 and continuing through July 1990. Much of the area sprayed was urbanized and included large population centers. Public concern about the health risks of these aerial applications was high, despite reassurances by state and local health officials. In an effort to address this widespread community concern, state health officials cooperated to collaborate with the Los Angeles County Department of Health Services staff to study doses of malathion in local residents alleging exposure to the aerial sprays. This collaborative effort led to the recruitment of over 60 local residents and agricultural workers for a study in which malathion's acid metabolites in urine were monitored. Malathion metabolites in human urine are detectable at levels as low as a few parts per billion (*2–4*), thus having the potential to quantify exposure to microgram quantities of the pesticide.

The purpose of this chapter is to illustrate how PB-PK modeling can be used in an epidemiological study to estimate the total absorbed dose of malathion when coupled with internal-dose biomarker data, in this case urinary

malathion acid metabolite concentrations. To date, the development and validation of PB-PK models has largely been accomplished by toxicologists studying experimental animals. This simulation study is unique, however, in that it involves local residents and agricultural workers with probable exposures incurred under "real world" conditions.

Materials and Methods

Study Cases and Exposure Scenario. For the Medfly eradication campaign in southern California during 1989–1990, a 20% mixture of technical grade malathion (with 95% purity) diluted in a corn-syrup protein bait was applied over many nights by helicopter over several hundred square miles, at a rate of 2.3 oz active ingredient per acre (equivalent to approximately 2μg/cm^2). The aerial applications were found to be uniform, as the deposition measured on test cards placed throughout spray areas rarely varied by more than 50% of the application rate. Spray droplets ranged in diameter from about 100 μm up to about 1–2 mm. By the following morning, the sprayed droplets typically had dried and hardened and were difficult to dislodge from flat surfaces. Malathion concentrations in outdoor air ranged up to a few micrograms per cubic meter of air.

A total of 67 individuals participated in the Los Angeles biomonitoring study. These included 30 women (29 residents and 1 agricultural worker), 20 men (13 residents and 7 agricultural workers), and 17 children (8 girls and 9 boys). Participants (or parents of the children) recruited were asked during an interview to respond to a questionnaire about their age, gender, circumstances of exposure, duration of exposure, and prevailing symptoms. The general profile of the participants and their exposure experience were previously described in detail by Papanek and Woloshin (*5*). Participants were instructed to collect a urine specimen at home in any ordinary clean glass jar and to freeze the specimen immediately. They were also asked to label the specimens with the date and time of collection. The specimens were then collected by county health department staff, thawed in the cold, and transferred to uniform glass jars, which were frozen again. These refrozen specimens were later sent to the Hazardous Materials Laboratory (HML) of the California Department of Health Services in Berkeley for analysis. In addition, 24 specimens were split to provide the Pacific Toxicology Laboratories in Los Angeles with duplicates to be analyzed for quality control purposes.

For the case study described here, only the 11 subjects with detectable malathion acid metabolites in their urine were included for PB-PK simulation. These 11 study cases, together with their estimated level of total acid metabolites (i.e., MCA + DCA), are listed in Table I. These 11 subjects all reported either to have been outdoors at night directly under an aerial application of the malathion bait or to have had extensive and direct skin con-

Table I. Excretion of Urinary Malathion Acid Metabolites by Subjects Potentially Exposed to Medfly Eradication Sprays[a]

Subject[b]	*Case ID*	*Age*	*Creatinine (g/L urine)*	*Acid Metabolites (μg/L urine)*[c]
Adult residents (70 kg)				
A	03	60	1.7	20.7
B	04	48	2.1	81.0
C	39	60	1.9	21.6
D	63	51	1.2	147.0
Agricultural workers (70 kg)				
E	AG49	39	1.2	45.0
F	AG52	45	1.3	81.0
G	AG53	57	1.0	40.0[d]
Younger children (14 kg)				
H	48	2	0.2	225.0
I	61	3	1.4	24.9
Older children (35 kg)				
J	31	10	2.3	75.0
K	60	5	1.2	156.0

[a]Presumably from direct spray or skin contact.
[b]The time lapse from first dermal contact until urine collection was reported to be within 12 h for all study cases, except subjects B, C, and D; according to their questionnaire, the time lapses for subjects B, C, and D were within 24–36 h, 12–36 h, and 36–48 h, respectively.
[c]Based on the dicarboxylic acid metabolites measured by the HML of the California Department of Health Services; total mono- and dicarboxylic acid metabolites were estimated to be three times the amount of dicarboxylic acid metabolites measured.
[d]Based on the total mono- and dicarboxylic acid metabolites measured by the Pacific Toxicology Laboratories in Los Angeles, whose analytical results were used primarily for quality-control purposes.

tact with a sprayed surface, such as grass or backyard foliage. All other subjects with less exposure, such as those merely residing in or walking through a sprayed area, were found to have undetectable levels of urinary metabolites. Also included in Table I are the urinary creatinine levels and subjects' ages. As mentioned in the table footnote, only DCA was measured by HML. The urine samples were collected and analyzed before a commitment was made to estimate also the absorbed doses through PB-PK simulation, which at this time can make use of only the total acid metabolites since the required metabolic rate constants (V_{max}) and Michaelis constant (K_M) for simulation are unavailable for the individual DCA or MCA. The MCA for each of the

tabolites by Subjects
in Sprays[a]

ne e)	*Acid Metabolites (μg/L urine)*[c]
	20.7
	81.0
	21.6
	147.0
	45.0
	81.0
	40.0[d]
	225.0
	24.9
	75.0
	156.0

n was reported to be within
g to their questionnaire, the
-36 h, and 36–48 h, respec-

HML of the California De-
metabolites were estimated
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easured by the Pacific Toxi-
e used primarily for quality-

d foliage. All other sub-
g in or walking through
vels of urinary metabo-
ine levels and subjects'
was measured by HML.
fore a commitment was
B-PK simulation, which
olites since the required
ant (K_M) for simulation
e MCA for each of the

11 study cases thus was estimated as two times that of the measured DCA in order to account for the total acid metabolites excreted.

According to Bradway and Shafik (2), over half of the malathion metabolites excreted in human urine appear in the form of MCA or DCA. A malathion clearance study conducted recently by the California Department of Pesticide Regulation (DPR) also indicated that the ratio of MCA to DCA metabolites in human urine changed over time (6). This ratio ranged from approximately 3:1 at 6-h to 1:1 at 12 h following exposure, which suggested sequential deesterification. The MCA-to-DCA ratio used here was considered to be valid, in that the mean MCA (provided by the reference laboratory) among the quality control samples was approximately 1.9 times higher than their mean DCA.

PB-PK Simulation. For the past decade, numerous investigators have used PB-PK models to predict tissue dose in animals and humans (7–25). These models can be highly isomorphic with the physiological and biochemical system of a specific mammalian species and thus may be useful to both toxicologists and epidemiologists. PB-PK models are defined by a set of mathematical equations used to simulate the time course of a chemical's disposition in several preselected tissue compartments. Each compartment has its own characteristic blood flow, volume, tissue-blood partition coefficient, and metabolic- or clearance-rate constants that together explain the chemical's disposition in that region. A comprehensive discussion of the details of constructing PB-PK models has been provided by Bischoff (26), Gibaldi and Perrier (27), and Andersen (28). The two basic steps in the construction of a PB-PK model are (1) choice of body regions and (2) postulation of a set of mathematical equations that will adequately relate the chemical's disposition in the preselected regions.

The basic structure of a PB-PK model for dermal exposure is outlined in Figure 1. The dermal-exposure model in Figure 1 shows that there is a series of mass-balance differential equations needed to account for the time course of the chemical's disposition occurring in the tissue compartments. The model assumes that where the skin is exposed to a chemical, a portion diffuses into the skin, and the rest is either lost to the atmosphere (as by evaporation) or remains on the skin. The portion that has been absorbed into the skin is further assumed to be gradually distributed to the various tissue compartments via circulation. In general, the amount in a particular tissue compartment is affected by many of the following biological variables: (1) the amount of the chemical available in the circulation at the time in question; (2) the cardiac output and the blood flow to the tissue involved; (3) the rates of clearance and metabolism, if they take place; and (4) the partition coefficient between the tissue and the blood involved, which is also defined as the solubility or concentration of the chemical in the tissue, com-

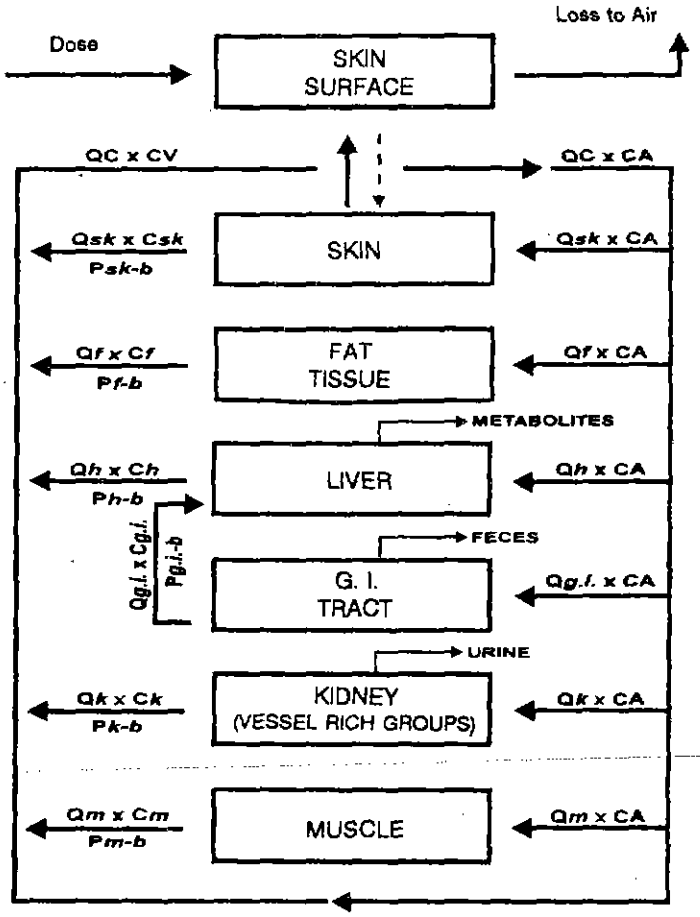
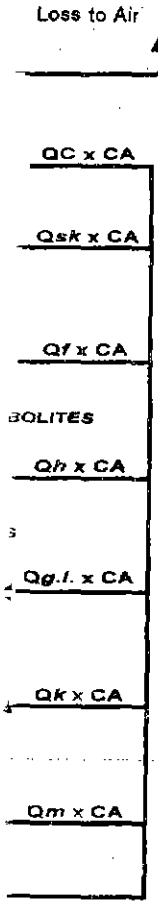


Figure 1. A typical PB-PK model for dermal exposure (see box for explanation of notations used).

Qi is blood flow to tissue i, Pi – b is tissue i – blood partition coefficient; Ci is concentration in tissue i; QC is cardiac output, CV is mixed venous concentration, CA is mixed arterial concentration, and CA is equal to CV.

pared with that in the blood at a steady state. The variables in the last two categories are highly specific to the chemical under investigation.

Many of the differential equations that are typically used in a model for dermal exposure (*see* box) have been used in the PB-PK models for dermal absorption of pesticides by Knaak et al. (*20*) and of organic vapors by McDougal et al. (*9, 21*). Equation 6 relies on the widely accepted assumption



see *box for explana-*

coefficient; Ci is con-
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vestigation.
v used in a model for
K models for dermal
ganic vapors by Mc-
accepted assumption

that venous liver concentrations, rather than liver concentrations, are used to derive the V_{max} and K_M values involved. The kinetic equations listed in the box consist of nonlinear terms and hence cannot be directly integrated. They can be solved indiscriminately, however, with numerical procedures that have been written to approximate an analytical solution.

One numerical procedure that has been considered to offer the most accurate (but also more laborious) integration approximation is the Runge–Kutta method, the computational details of which are readily available in many modern elementary textbooks on differential equations. The algorithm for this numerical procedure can be implemented with any computer programming language such as FORTRAN, BASIC(A), or PASCAL. Within DPR, this algorithm has been written in BASIC(A) for PB-PK simulation on an IBM-compatible microcomputer (29). More sophisticated simulation packages, such as SimuSolv, SCoP, and STELLA, are also available for solving differential equations formulated by the analyst. To date, however, no commercially available simulation programs have been written specifically for PB-PK simulation of dermal exposure.

Table II lists all the physiological and biochemical values that were used in the PB-PK model constructed here specifically for dermal exposure to malathion. Many of these parameter values were also used in two earlier related studies by the California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (OEHHA), where justification for their use was given (*30*, *31*). Further elaboration on the use of these values is provided in the next section. In addition to equation 6 noted earlier, another modification made here to the OEHHA model was the assumption that malathion leaving the gastrointestinal tract compartment would circulate into the liver compartment. This additional modification is consistent with common practice in which the gastrointestinal tract is treated as a separate compartment and is reflected in Figure 1 and in equation 7.

The objective of this PB-PK modeling was to simulate model predictions of the cumulative urinary excretion of malathion acid metabolites to the amounts equivalent to those estimated from the biomonitoring data. Once there was a good approximation (match) between the simulated and the observed values, the total absorbed dose of malathion would be calculated by summing the amounts simulated for all internal tissues, including those remaining in the skin and those excreted. The observed urinary excretions for the 11 study cases were first calculated for a 24-h accumulation by multiplying the estimated urinary level of acid metabolites in Table I by the assumed daily urine output volume. The 24-h accumulation was then adjusted for the two (shortest and longest) times from exposure to urine collection assumed for each study case. The daily urine output volumes for the adults, younger children, and older children were assumed to be 1200, 500, and 800 mL, respectively (*32*). Because the creatinine levels measured for the study cases indicate that the daily urine output volumes tended to be overestimated

Equations Typically Used in a PB-PK Model for Dermal Exposure[a]

$$CA = \{(Q_{sk} \times C_{sk}/P_{sk\text{-}b}) + (Q_f \times C_f/P_{f\text{-}b}) + (Q_h \times C_h/P_{h\text{-}b}) + (Q_k \times C_k/P_{k\text{-}b}) + (Q_m \times C_m/P_{m\text{-}b})\}/QC \quad (1)$$

Mixed arterial (CA) concentration is the sum of the amounts eliminated by the individual compartments divided by the cardiac output QC (i.e., by the sum of the individual Q_i). The amount eliminated in each compartment *i* is denoted by $Q_i \times C_i/P_{i\text{-}b}$, where Q_i = blood flow to tissue *i*, C_i = concentration in tissue *i*, and $P_{i\text{-}b}$ = tissue *i*/blood partition coefficient. (Throughout this box, the following notations are used: sk = skin; f = fat; h = hepatic; k = kidney; g.i. = gastrointestinal tract; m = muscle; surf = skin surface; met = metabolite; u = urine; fec = feces; and AMT = amount.)

$$dAMT_{surf}/dt = K_{sp} \times A \times (C_{sk}/P_{sk\text{-}a} - C_{exp}) - K_a \times AMT_{surf} \quad (2)$$

The amount *on* the skin (AMT_{surf}) changes over time as a function of three events: (1) the amount diffused from *inside* to *outside* of the skin (*although at times this amount is negligible*); (2) the amount absorbed *into* the skin; and (3) the amount lost to the air. (K_{sp} = skin permeability constant; A = skin surface area exposed; $P_{sk\text{-}a}$ = skin–air partition coefficient; K_a = evaporation constant; and C_{exp} = concentration of dose applied *topically*.) (Where C_{exp} is averaged *air* concentration, both this and Equation 3 will no longer be applicable, because in that case the applied dose will not diminish over time.)

$$dAMT_{air}/dt = K_a \times AMT_{surf} \quad (3)$$

The amount lost to the air is a function of $K_a \times AMT_{surf}$

$$dAMT_{sk}/dt = K_{sp} \times A \times (C_{exp} - C_{sk}/P_{sk\text{-}a}) + Q_{sk} \times (CA - C_{sk}/P_{sk\text{-}b}) \quad (4)$$

The amount absorbed into the skin is a function of three events: (1) the amount diffused *into* the skin; (2) the amount diffused from *inside* to *outside* of the skin; and (3) the amount of difference between that perfused to and that eliminated in the skin tissue.

$$dAMT_f/dt = Q_f \times (CA - C_f/P_{f\text{-}b}) \quad (5)$$

The amount in fat is related directly to the amount of difference between that perfused to and that eliminated in the fat tissue.

$$dAMT_{met}/dt = (V_{max} \times C_h)/\{(K_M \times P_{h\text{-}b}) + C_h\} \quad (6)$$

This metabolism rate is based on the well-known Michaelis–Menten equation; for some chemicals, this equation may occur in a tissue organ other than the hepatic system or may take another form, such as a first-order reaction.

Dermal Exposure[a]

(1)

iounts eliminated by the
ut QC (i.e., by the sum
mpartment *i* is denoted
= concentration in tissue
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= hepatic; k = kidney;
surface; met = metab-

AMT_{surf} (2)

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of the skin (*although at*
ed *into* the skin; and (3)
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= evaporation constant;
(Where C_{exp} is averaged
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over time.)

(3)

surf

$CA - C_{sk}/P_{sk\text{-}b})$ (4)

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(5)

f difference between that

(6)

haelis–Menten equation;
sue organ other than the
irst-order reaction.

Equations Typically Used in a PB-PK Model for Dermal Exposure[a]—*Continued.*

$$dAMT_b/dt = Q_b \times (CA - C_b/P_{b\text{-}b}) + (Q_{g.i} \times C_{g.i}/P_{g.i\text{-}b}) - dAMT_{met}/dt \quad (7)$$

$$dAMT_u/dt = K_u \times AMT_k \qquad (K_u = \text{urinary constant}) \quad (8)$$

$$dAMT_k/dt = Q_k \times (CA - C_k/P_{k\text{-}b}) - K_u \times AMT_k \quad (9)$$

$$dAMT_{g.i}/dt = Q_{g.i} \times (CA - C_{g.i}/P_{g.i\text{-}b}) - K_{fec} \times GI \qquad (K_{fec} = \text{fecal constant}) \quad (10)$$

$$dAMT_{fec}/dt = K_{fec} \times GI \quad (11)$$

$$dAMT_m/dt = Q_m \times (CA - C_m/P_{m\text{-}b}) \quad (12)$$

[a]These equations are summarized graphically in Figure 1. Equations 7 through 12 are not elaborated here, because on reviewing the first few equations, the reader should find their interpretations all to be repetitive. For dermal exposure, mixed venous concentration (as denoted by CV in Figure 1) is assumed to be approximately equal to CA.

(perhaps with the exception for the agricultural workers), their 24-h accumulations and absorbed doses were likely to be overestimated as well. Several investigators (33, 34) have recently found the excretion rate of urinary creatinine to change over time. It was primarily because of these observations that the creatinine levels were not used here to calculate the daily urine output volumes.

Results and Discussion

The amounts of malathion and of its acid metabolites simulated for the various tissue groups for subject B are presented in Table III. These results exemplify the total absorbed dose of malathion simulated for the longest assumed time lapse from exposure to urine collection for subject B, whose observed malathion acid metabolites in urine accumulated up to 36 h were estimated to be (81 μg/L × 1.2 L/24 h × 36 h =) 145.8 μg (*see* footnotes in Table I and the box for assumptions). As shown in Table III, the accumulation of acid metabolites simulated at 36 h was 145.79 μg. In order for the body to produce and excrete the acid metabolites in this amount in 36 h under the physiological and biochemical constraints specified by the model, the amounts of malathion and its metabolites accumulated in the various tissue compartments should approximate those specified in the table. The total absorbed dose of malathion for this case was estimated to be 0.56 mg, which is simply the sum of the amounts of malathion and of its acid

Table II. Typical Physiological and Biochemical Values Used in PB-BK Human Models for Dermal Exposure to Malathion[a]

Parameters	*Adult (70 kg)*	*Younger Child (14 kg)*	*Older Child (35 kg)*
A. Tissue volumes (L)			
Fat	10.0	2.0	5.0
Intestine	2.4	0.48	1.2
Kidney[b]	2.7	0.54	1.35
Liver	1.5	0.30	0.75
Muscle	30.0	6.0	15.0
Skin	2.6	0.52	1.3
B. Tissue perfusion rates (L/min)			
Fat	0.2	0.06	0.12
Intestine	1.2	0.36	0.71
Kidney[b]	2.25	0.68	1.34
Liver	1.5	0.45	0.89
Muscle	1.2	0.36	0.71
Skin	0.125	0.04	0.07
Cardiac output	6.475	1.95	3.84
C. Hydrolytic liver metabolism[c]			
V_{max} (mole/min)	4.89×10^{-4}	1.46×10^{-4}	2.91×10^{-4}
K_m (mole/L)	1.35×10^{-4}	1.35×10^{-4}	1.35×10^{-4}
D. Other kinetic parameters (min^{-1})			
Skin permeability constant	1.0×10^{-4} 1.0×10^{-4}	1.0×10^{-4} 1.0×10^{-4}	1.0×10^{-4}
Evaporation constant	20.0 0.1	20.0 0.1	1.0×10^{-4}
Urinary constant			20.0
Fecal constant			0.1
E. Tissue/blood partition coefficient			
Fat	775.0		
Intestine	15.0		
Kidney[b]	17.0		
Liver	33.6		
Muscle	22.8		
Skin	25.0		

[a]Based on those adopted by the California Environmental Protection Agency OEHHA (*30, 31*).
[b]Including other vessel rich groups (brain, heart, and lungs).
[c]Available only for total mono- and dicarboxylic acid metabolites.

s Used in PB-BK Human
athion[a]

r Child kg)	Older Child (35 kg)
	5.0
	1.2
	1.35
	0.75
	15.0
	1.3
	0.12
	0.71
	1.34
	0.89
	0.71
	0.07
	3.84
10^{-4}	2.91×10^{-4}
10^{-4}	1.35×10^{-4}
10^{-4}	1.0×10^{-4}
10^{-4}	1.0×10^{-4}
	20.0
	0.1

ection Agency OEHHA (30.

metabolites simulated for all internal tissues at any time after 8 h from first dermal contact (after the dermal dose presumably was washed off).

In Table III, the disposition of malathion in the various tissue compartments for subject B was for preselected hourly intervals only. The PB-PK model actually simulated the amount of chemical in each tissue at 0.1-min (6-sec) intervals. The output American Standard Code for Information Interchange (ASCII) file from the computer program, on the other hand, listed these serial amounts at every 10-min interval. Although in Table III the amounts listed for some of the tissues decreased over time as a result of redistribution, all listed amounts actually represented an accumulation or an account of the chemical or its metabolites available up to the specified time interval, rather than that present at the specified time. Figure 2 provides a graphic view of this output for the acid and other metabolites excreted by subject B. As shown in Figure 2, the ratio of acid metabolites to other malathion metabolites for this individual was approximately 1:2. In addition to their (cumulative) total, the excretion rate of malathion acid metabolites may also be determined from the simulation data listed in the output file. The excretion rates of malathion acid metabolites calculated at various time intervals for subject B are depicted in Figure 3. As shown, the excretion rate peaked at approximately 9 h after first dermal contact.

The total absorbed doses of malathion simulated for the 11 study cases are summarized in Table IV. This summary table shows that subject D experienced the highest dose, although this individual did not have the highest level of urinary metabolites. This simulation result was not inconsistent with general expectation, however, in that the subject's daily urine output was assumed to be 1200 mL. Because of age difference, the daily urine outputs for subjects H and K were assumed to be 500 and 800 mL, respectively. Thus, on a cumulative basis, subject D should have higher urinary acid metabolite output (which would lead to the estimation of a higher absorbed dose), even though subjects H and K had the highest metabolite levels detected.

Overall, the result simulated from this PB-PK modeling were found to be consistent with those available in the literature. In this case study, the acid metabolites in the liver were assumed to be excreted primarily into the urine shortly after they were produced. According to Bradway and Shafik (2), MCA and DCA excreted in human urine could account for 57% of the total urinary malathion metabolites. Table III suggests that the acid metabolites accumulated through simulation were approximately 36% of the total malathion metabolites excreted in urine. Although there appeared to be a significant difference between the two studies in the percentage of acid metabolites excreted, the lower percentage of acid metabolites obtained from this PB-PK simulation was not unexpected. The PB-PK model constructed here was for dermal exposure, whereas the observation by Bradway and Shafik involved oral exposure based on a single suicide case. As can be seen

Table III. PB-PK Simulation of Amounts of Malathion in Tissues for Subject B over a One-Week (168-h) Period[a]

Hours Since Contact[b]	*On Skin*	*To Air*	*In Skin*	*Fat*	*Liver*	*GI Tract*	*Feces*	*Kidney*	*Urine*	*Muscle*	*Acid Metabolites*[c]
0	13.486	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	13.009	0.397	75.125	0.146	0.351	0.191	0.516	0.003	1.641	0.849	0.650
2	12.549	0.781	139.573	0.568	0.743	0.389	2.269	0.005	6.380	3.183	3.027
3	12.105	1.150	194.581	1.232	1.086	0.562	5.131	0.007	13.840	6.663	6.986
4	11.677	1.507	241.250	2.106	1.384	0.712	8.963	0.009	23.676	10.999	12.327
5	11.264	1.851	280.560	3.162	1.643	0.842	13.636	0.011	35.572	15.947	18.868
6	10.866	2.183	313.382	4.375	1.866	0.955	19.036	0.012	49.249	21.302	26.447
7	10.482	2.503	340.491	5.722	2.057	1.051	25.061	0.013	64.452	26.895	34.918
8	10.111	2.812	362.576	7.183	2.219	1.132	31.617	0.014	80.954	32.588	44.148
9	0.000	2.817	324.828	8.632	2.097	1.060	38.250	0.013	97.361	37.651	53.551
10	0.000	2.817	290.217	9.954	1.916	0.968	44.331	0.012	112.356	41.492	62.199
11	0.000	2.817	259.310	11.158	1.751	0.885	49.886	0.011	126.055	44.292	70.099
12	0.000	2.817	231.709	12.255	1.600	0.808	54.962	0.010	138.573	46.214	77.317
24	0.000	2.817	60.377	19.794	0.551	0.278	90.681	0.003	226.665	37.001	128.108
36	0.000	2.817	15.942	22.134	0.195	0.099	103.120	0.001	257.354	18.823	145.794
48	0.000	2.817	4.288	22.695	0.071	0.036	107.574	0.000	268.345	8.326	152.126
60	0.000	2.817	1.184	22.637	0.027	0.014	109.227	0.000	272.426	3.475	154.474
72	0.000	2.817	0.340	22.363	0.011	0.006	109.879	0.000	274.035	1.429	155.401
84	0.000	2.817	0.105	22.014	0.006	0.003	110.168	0.000	271.752	0.606	155.812
96	0.000	2.817	0.037	21.642	0.003	0.002	110.324	0.000	275.154	0.283	156.038
108	0.000	2.817	0.017	21.266	0.003	0.001	110.431	0.000	275.369	0.158	156.171
120	0.000	2.817	0.011	20.891	0.002	0.001	110.538	0.000	275.584	0.110	156.278
132	0.000	2.817	0.009	20.526	0.002	0.001	110.605	0.000	275.798	0.091	156.385
144	0.000	2.817	0.008	20.164	0.002	0.001	110.659	0.000	276.013	0.082	156.493
156	0.000	2.817	0.007	19.809	0.002	0.001	110.712	0.000	276.227	0.079	156.600
168	0.000	2.817	0.007	19.460	0.002	0.001	110.766	0.000	276.442	0.076	156.707

[a]Based on long time lapse since first dermal contact until urine collection for subject B, whose observed urinary malathion acid metabolites accumulated up to 36 h were estimated to be 145.8 μg; all amounts, except for those remaining on the skin (mg) and evaporated to the air (mg), are expressed in micrograms; amounts in blood were negligible compared with those in other tissues and hence were not listed here.
[b]Under the assumption that the applied dose would be washed off 8 h after first dermal contact (*see* text for discussion on the use of applied dose).
[c]Includes both mono- and dicarboxylic acids.

96	0.000	2.817	0.017	21.642	0.003	0.002	110.324	0.000	276.134	0.253	156.057
108	0.000	2.817	0.017	21.266	0.003	0.001	110.431	0.000	275.369	0.158	156.171
120	0.000	2.817	0.011	20.891	0.002	0.001	110.538	0.000	275.584	0.110	156.278
132	0.000	2.817	0.009	20.526	0.002	0.001	110.605	0.000	275.798	0.091	156.385
144	0.000	2.817	0.008	20.164	0.002	0.001	110.659	0.000	276.013	0.082	156.493
156	0.000	2.817	0.007	19.809	0.002	0.001	110.712	0.000	276.227	0.079	156.600
168	0.000	2.817	0.007	19.460	0.002	0.001	110.766	0.000	276.442	0.076	156.707

[a]Based on long time lapse since first dermal contact until urine collection for subject B, whose observed urinary malathion acid metabolites accumulated up to 36 h were estimated to be 145.8 μg; all amounts, except for those remaining on the skin (mg) and evaporated to the air (mg), are expressed in micrograms; amounts in blood were negligible compared with those in other tissues and hence were not listed here.
[b]Under the assumption that the applied dose would be washed off 8 h after first dermal contact (*see* text for discussion on the use of applied dose).
[c]Includes both mono- and dicarboxylic acids.

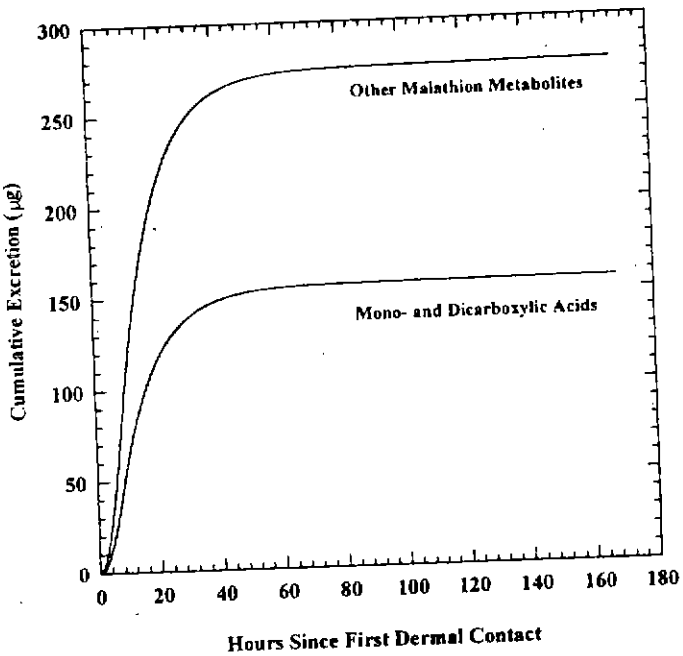

Figure 2. Excretion of urinary malathion metabolites (simulated for subject B).

in Figure 1, a chemical absorbed in the gastrointestinal tract is likely to be immediately available in the liver, the dominant site for chemical metabolism (including that for malathion). However, the same chemical from dermal exposure is likely to be more available to other tissue compartments and hence could be excreted without first being metabolized into MCA or DCA in the liver. Carboxylesterase is responsible for the degradation (deesterification) of malathion into the acid metabolites. It has been shown (35–37), however, that in the general population the level of circulating carboxylesterase available in the blood is negligible compared with that in the liver.

Table III also suggests that the excretion half-time of malathion was approximately 12 h after first dermal contact, which was assumed to have lasted 8 h. At 12 h, the total amount of excretion shown in Table III (i.e., that listed under feces, urine, and acid metabolites) was 0.27 mg, approximately half of the total absorbed dose estimated (for long-time lapse). This finding was consistent with that observed earlier in the study by Ross et al. (6), in which a range from 4 to 12 h was reported as the excretion half-time of malathion applied to the skin in a vehicle (which included protein bait). The excretion data in Table III indicate that at 48 h after first dermal contact, more than 75% of an absorbed dose would be recovered as urinary (acid and other) metabolites with another 20% recovered as fecal metabolites. The urinary

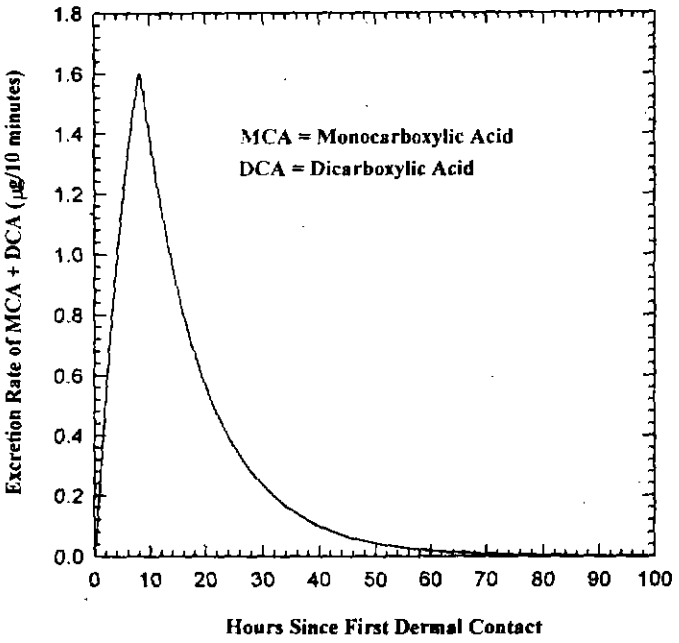

Figure 3. Excretion curve for malathion acid metabolites (simulated for subject B).

recovery simulated in this study was only 15–20% lower than those observed experimentally elsewhere (*6*, *38*). The excretion rates simulated here for malathion acid metabolites (as shown in Figure 3) were similar to those estimated independently by Papanek and Woloshin (*5*) and those observed by others for total malathion metabolites (*38*, *39*), thus further substantiating the kinetic equations used in the model.

Absorbed doses of malathion estimated here through PB-PK simulation were typically within an order of magnitude of the upper-bound estimates calculated earlier by Papanek and Woloshin (*5*). In that exposure assessment, relying on a different computational method and largely a different set of assumptions, Papanek and Woloshin also found the highest total absorbed doses (3.3 mg for short lapse and 9.6 mg for long lapse) in subject D. The lowest absorbed doses (30 μg for short lapse and 9 μg for long lapse) estimated in their exposure assessment also occurred in subject I. As shown in Table IV, the difference in time lapse tended to have an impact on the estimation of total absorbed dose.

In general, model predictions are greatly affected by the input parameters. Although many of the physiological and biochemical parameters applied in the PB-PK model were also used earlier by OEHHA (*30*, *31*), it is

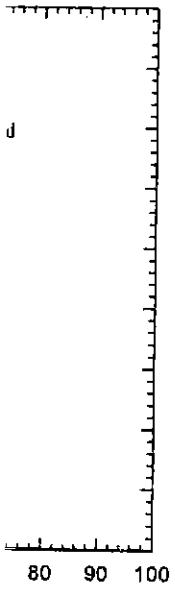

Table IV. Total Absorbed Dose of Malathion Estimated through Modeling

Subject	*Case ID*	*Acid Metabolites[a] (μg/L Urine)*	*Malathion Dose (mg)[b]*	
			Short Lapse	*Long Lapse*
Adult residents (70 kg)				
A	03	20.7	0.11	0.09
B	04	81.0	0.43	0.56
C	39	21.6	0.10	0.15
D	63	147.0	1.00	1.31
Agricultural workers (70 kg)				
E	AG49	45.0	0.22	0.20
F	AG52	81.0	0.40	0.36
G	AG53	40.0	0.20	0.18
Younger children (14 kg)				
H	48	225.0	0.30	0.30
I	61	24.9	0.03	0.03
Older children (35 kg)				
J	31	75.0	0.20	0.19
K	60	156.0	0.42[c]	0.40[c]

[a]The amount of total acid metabolites accumulated up to the assumed time lapse between first dermal contact and urine collection (*see* Table I) was used to simulate each participant's total absorbed dose of malathion; the daily urine output volumes for adults, younger children, and older children were assumed to be 1200, 500, and 800 mL, respectively (32).
[b]Each participant's dose was also simulated by using the shortest as well as the longest assumed number of hours between first dermal contact and urine collection; for all study cases, except subjects B, C, and D, the shortest time lapse was assumed to be 3 h (*see* footnotes in Table I for assumed time lapses).
[c]May be substantially overestimated because of overestimation of the child's body weight (32); as shown in Table I, the child was 5 years old at the time of the biomonitoring study.

conceivable that the input parameters listed in Table II might be best defined by their ranges, rather than by their means or mean-based point estimates. A more reliable and more complex approach to PB-PK modeling would thus be to use parameter values randomly sampled from their range, while taking into account their known or presumed statistical distribution. Many investigators have begun to utilize this approach to refining or improving their PB-PK models in risk assessment (*14–16, 23, 24*). For this approach, Monte Carlo simulations would also be required to obtain a representative sample of model predictions, which by definition would be statistical distributions rather than single points.

The Monte Carlo technique was not incorporated into the PB-PK modeling here, however, despite its increasing popularity. A major reason for this omission was that other parameters used in the model had even greater uncertainty. These included the assumed time lapse and the estimated total acid metabolites estimated for the 11 study cases. As shown in Table IV, dose simulation was affected somewhat by the assumed time lapse. A great deal of uncertainty was associated with this time variable, because its measurement was primarily based on the participant's recollection. The values estimated for the observed total acid metabolites were also subject to substantial uncertainty, in that direct measurements were not made on MCA and total urine outputs were not collected for the participants. Because the purpose of this case study was illustrative and the biomonitoring study had limitations in its experimental design, the complex Monte Carlo approach to PB-PK modeling was not warranted here. (For a limited discussion of improved experimental design *see* the Conclusion section.)

Although the Monte Carlo approach was not used in this case study, over 70 additional simulations were made to determine the sensitivity of dose estimation to the key biochemical parameters listed in Table II (for all subjects). When the partition coefficient value for any of the listed tissues other than skin was deliberately doubled, the absorbed dose predicted was altered by about 15% or less. Even when either the V_{max} or K_M value was doubled, the dose was altered by no more than 35%. In this sensitivity analysis, model predictions were found to be most affected (up to as much as two-fold) by the skin–blood partition coefficient. Such a finding was not unexpected, however, because the tissue–blood partition coefficient is defined as the concentration of a chemical in the tissue compared with that in the blood at steady state. If more malathion were bound in the skin (as would be the case with an increased partition coefficient), less would be available to the liver for metabolism. This effect, in turn, would require an additional dose absorbed into the body (the skin) so that the same theoretical amount of malathion could be available to the liver for metabolism.

Several input parameters listed in Table II were assigned arbitrary values. These parameters included the constants for skin permeability and for evaporation into air. In this case study, the total absorbed dose (not the applied dermal dose) was of interest. The PB-PK model was constructed for a dose applied to a skin surface area of *1 cm*^{2}. The applied dose was at first assigned an arbitrary value for a given study case and then was varied in subsequent runs until the predicted accumulation of acid metabolites matched that estimated from the biomonitoring data. In short, the model was independent of the total exposed surface area. If the actual exposed surface area were 1000 cm^2, the model dose corresponding to a 1-cm^2 surface area would need to be reduced by 1000-fold (so that the total dermal dose that would penetrate through the skin would remain the same). This prop-

osition is in accord with the following equation given by McDougal et al. (*21*) and by Knaak et al. (*25*):

$$ABS = P \times A \times C \times T$$

where P is the skin permeability constant, A is the surface area exposed, C is the exposure concentration, T is the permeation time, and ABS is the total amount of chemical absorbed up to permeation time T. An extension of this equation made here is that ABS is quantifiable by summing the amounts of chemical simulated for all the internal tissue compartments involved. The amount of chemical in each compartment is estimated by the postulated kinetic equations so that the total comprises the ABS. Although the amounts of chemical in the internal tissues change over time, their sum after permeation stops (in this case after 8 h) always remains the same; this is because the chemical (or its metabolites) has to be present in some of the internal tissue compartments. This assurance of additivity is based on the commonly encountered biochemical principle known as the mass-balance theory.

Unlike most air levels of organic vapors assumed for dermal exposure assessment, the total applied malathion dose (i.e., $A \times C$) received through dermal contact by each subject in this case study was expected to dissipate over time. As reflected in equation 2, its quantity on the skin diminishes over time because of permeation and evaporation. Insofar as the constants for skin permeability and evaporation are comparatively small and their ratio does not alter substantially, the applied dose that remains on the skin will dissipate at a relatively very low, fairly steady rate (for an example, *see* Table III). Consequently, the absorbed dose simulated here should not be affected by the constants assumed for skin permeability or for evaporation, unless these assigned values, particularly that for the latter constant, are far off the range limits proposed by OEHHA (*30*, *31*).

Conclusion

As discussed in several earlier chapters in this volume, classification of exposure groups is a cornerstone of well-designed studies in environmental epidemiology. The increasing sensitivity and sophistication of analytical tests have greatly expanded the array of biomarkers available to the epidemiologist. Data on biomarkers in biological fluids, regardless of how accurate, usually do not correspond in any direct way to the dose absorbed. As demonstrated in this chapter, PB-PK models provide an essential interpretive tool when applied to biomarker data.

PB-PK modeling was used in this case study to estimate the total absorbed dose of malathion in seven adults and four children. The subjects were alleged to have come into contact with the Medfly eradication aerial

sprays that were applied over portions of southern California during the fall of 1989 and continuing through July 1990. Results predicted through this PB-PK simulation suggest that for the adults (70 kg), the highest absorbed dose of malathion was 1.3 mg in the spray event studied. Among the younger (14 kg) and the older (35 kg) children, the highest estimated total doses were 0.3 mg and 0.4 mg, respectively. These estimations were based on a conservative approach, in that the model assumed an above-average daily urine output for the study cases and considered the shortest as well as the longest probable time lapse from first dermal contact until urine collection. In addition, the model assumed that as much as 20% of an absorbed dose of malathion would be excreted in feces, thus resulting in a lower production of the acid metabolites that would lead to a higher quantity of the *ABS* simulated.

In addition to its utility in estimating the total absorbed dose of a chemical, PB-PK simulation can be used to estimate simultaneously the various time-dependent tissue concentrations. The amounts of malathion or its metabolites simulated for the various internal tissues shown in Table III, when divided by their corresponding tissue volume, would provide the tissue concentrations of interest. This type of pharmacokinetic information is crucial for health risk assessment if the toxic end point in question is organ-specific. Gearhart et al. (*18*) recently demonstrated the use of PB-PK models for predicting cholinesterase inhibition in rats. Although malathion can cause acute cholinergic reactions in humans at high dosages, the PB-PK model used in this case study was not revised to separate brain from the vessel-rich groups. This is because the objective here was not to estimate the various tissue concentrations per se but rather to estimate total dosage.

Future studies need experimental design considerations to exploit more fully the sophistication and precision of PB-PK models. For example, analytical methods are needed to quantify the breadth of known malathion urinary metabolites, not just DCA or the sum of DCA and MCA. The PB-PK simulations performed would have been more reliable had the theoretical accumulation of malathion urinary metabolites (for each study case) been fit simultaneously as well as statistically to a set of experimental values corresponding to definite time points, such as at 6, 12, 24, and 48 h. Finally, additional information is needed on the background exposures to malathion in the general population. Confounding due to dietary exposures was not evaluated, other than by a questionnaire. Nevertheless, this pilot study was believed to be useful in providing a rough estimate of the total absorbed dose of malathion for individuals allegedly exposed to the Medfly eradication aerial sprays, particularly in light of the fact that the subjects were recruited largely from the general population in sprayed areas and that recruitment was based primarily on self-reported, alleged exposures surfacing in complaints to local health authorities.

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RECEIVED for review November 2, 1992. ACCEPTED revised manuscript January 28, 1993.